Pharmacology Biochemistry & Behavior, Vol. 14, pp. 889–893, 1981. Printed in the U.S.A.

Peripheral Conversion of *L*-5-Hydroxytryptophan to Serotonin Induces Drinking in Rats[1]

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Received 22 December 1980

KIKTA, D. C., R. M. THREATTE, C. C. BARNEY, M. J. FREGLY AND J. E. GREENLEAF. *Peripheral conversion of l-5-hydroxytryptophan to serotonin induces drinking in rats.* PHARMAC. BIOCHEM. BEHAV. **14**(6) 889–893, 1981.—Female rats administered serotonin (0.25 to 4.0 mg/kg, s.c.) showed a dose-dependent increase in water intake. The dipsogenic response was nearly maximal when 2 mg/kg was administered s.c. and plateaued by 2 hr after treatment. *l*-5-Hydroxytryptophan (5-HTP), the precursor of serotonin, is also a potent dipsogen which induces drinking by way of the renin-angiotensin system. The possibility that the dipsogenic activity of 5-HTP is dependent on decarboxylation to serotonin was the objective of these studies. Either benserazide (30 mg/kg, s.c.), a central and peripheral decarboxylase inhibitor, or carbidopa (6.5 mg/kg, s.c.), a peripheral decarboxylase inhibitor, was administered 15 min prior to the dipsogen. Both decarboxylase inhibitors attenuated the dipsogenic response to 5-HTP (25 mg/kg, s.c.) but not to serotonin (2 mg/kg, s.c.). The peripheral serotonergic receptor antagonist, methysergide (3 mg/kg, i.p.), blocked the dipsogenic responses to both 5-HTP (25 mg/kg, s.c.) and serotonin (2 mg/kg, s.c.). There was no interaction between 5-HTP (18 mg/kg, s.c.) and serotonin (1 mg/kg, s.c.) when administered simultaneously with respect to their dipsogenic effects. Thus, the drinking response accompanying administration of 5-HTP occurs following peripheral conversion to serotonin which, in turn, activates peripheral serotonergic receptors. The mechanism(s) by which activation of peripheral serotonergic receptors increases water intake is not known, but appears to involve release of renin from the kidney.

Drinking Serotonin *l*-5-Hydroxytryptophan Decarboxylase inhibitors Serotonergic receptors Benserazide Carbidopa Methysergide

MANY compounds have been found to induce drinking in rats [7]. Some dipsogenic agents increase water intake via the renin-angiotensin system [7]. Renin, released from the kidney, leads ultimately to the formation of angiotensin II, which is purported to induce drinking [8] through a central action [6]. Recently, studies from this laboratory have shown that *l*-5-hydroxytryptophan (5-HTP) is a potent dipsogen [10] which induces drinking [18] ultimately by increasing the release of renin from the kidneys [2]. 5-HTP is converted both peripherally and centrally to serotonin by a decarboxylase enzyme, *l*-5-hydroxytryptophan decarboxylase [13]. Recent evidence shows that serotonin acts centrally to increase renin release from the kidney of the dog [21]; therefore, the possibility exists that serotonin, rather than 5-HTP, is the dipsogenic agent.

The present study was designed to test the dipsogenic activity of serotonin in the rat and to determine whether 5-HTP must be converted, either centrally or peripherally, to serotonin in order to increase water intake. The effects of benserazide, a central and peripheral decarboxylase inhibitor [3], and carbidopa, a peripheral decarboxylase inhibitor [20], on the dipsogenic responses to 5-HTP and serotonin were studied. The effect of methysergide, a peripheral serotonergic receptor antagonist [1,9], on the drinking responses to 5-HTP and serotonin was also studied. The results indicate that 5-HTP is converted peripherally to serotonin which acts at peripheral serotonergic receptors to initiate an increase in water intake in the rat.

METHOD

Forty female rats of the Blue Spruce Farms (Hooded) strain weighing 220 to 310 g were used for this study. The rats were housed 4 per cage in a room maintained at 24±1°C and illuminated from 7 a.m. to 7 p.m. All rats were provided with Purina Laboratory Chow and tap water ad lib prior to all experiments.

[1]Supported by contract NCA2-OR204-101 from the National Aeronautics and Space Administration, Moffett Field, CA.
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For each experiment, the rats were divided randomly into either 4 or 6 groups of 6 rats each. At least 3 days were allowed between experiments. One hr prior to the beginning of an experiment, all rats were weighed and placed in individual stainless steel metabolism cages equipped with water bottles which consisted of infant nursing bottles with cast bronze spouts as described by Lazarow [12]. Food was not available during the experiments. All experiments began between 9 a.m. and 10 a.m. All compounds tested were dissolved in isotonic saline to give 1 ml/kg of body weight. Concentrations of serotonin and 5-HTP are not expressed as the base compound.

Experiment 1: Dose-Response Relationship Between Water Intake and Dose of Serotonin Administered Acutely

Six groups of rats were used in this experiment. Group 1 served as a control and received isotonic saline (1 ml/kg, s.c.). Groups 2–6 received serotonin at concentrations of 0.25, 0.5, 1.0, 2.0, and 4.0 mg/kg, s.c., respectively. All water bottles were weighed immediately before initiation of the study and were returned to the metabolism cages immediately following administration of either saline or serotonin. Water intake was measured 0.5, 1, 2, and 3 hr after administration of either saline or serotonin.

Experiment 2: Effect of Decarboxylase Inhibition on 5-HTP and Serotonin-Induced Water Intakes

Four tests were conducted for this experiment: (a) benserazide vs 5-HTP, (b) benserazide vs serotonin, (c) carbidopa vs 5-HTP, and (d) carbidopa vs serotonin. Four groups of rats were used in each test. Group 1 (control) received isotonic saline (1 ml/kg, s.c.) followed by isotonic saline (1 ml/kg, s.c.) 15 min later. Group 2 received a decarboxylase inhibitor, either benserazide (30 mg/kg, s.c.) or carbidopa (6.5 mg/kg, s.c.), followed by isotonic saline (1 ml/kg, s.c.) 15 min later. Group 3 received isotonic saline (1 ml/kg, s.c.) followed either by 5-HTP (25 mg/kg, s.c.) or by serotonin (2 mg/kg, s.c.) 15 min later. Group 4 received a decarboxylase inhibitor, either benserazide (30 mg/kg, s.c.) or carbidopa (6.5 mg/kg, s.c.), followed either by 5-HTP (25 mg/kg, s.c.) or by serotonin (2 mg/kg, s.c.) 15 min later. The water bottles were weighed prior to the second administration of drug and replaced immediately following the second administration. Water intake was then measured 1, 2, and 3 hr following the second administration of drug.

Experiment 3: Interaction Between 5-HTP and Serotonin on Induction of Drinking

Four groups of rats were used in this experiment. Group 1 (control) received simultaneously isotonic saline (1 ml/kg, s.c.) plus isotonic saline (1 ml/kg, s.c.). Group 2 received simultaneously serotonin (1 mg/kg, s.c.) plus isotonic saline (1 ml/kg, s.c.). Group 3 received simultaneously isotonic saline (1 ml/kg, s.c.) plus 5-HTP (18 mg/kg, s.c.). Group 4 received simultaneously serotonin (1 mg/kg, s.c.) plus 5-HTP (18 mg/kg, s.c.). Water intake was measured as described for Experiment 2.

Experiment 4: Effect of Methysergide on 5-HTP and Serotonin-Induced Water Intakes

Two tests were conducted for this experiment: (a) methysergide vs 5-HTP and (b) methysergide vs serotonin. Four groups of rats were used in each test. Group 1 (control) received isotonic saline (1 ml/kg, i.p.) followed by isotonic saline (1 ml/kg, s.c.) 15 min later. Group 2 received methysergide (3 mg/kg, i.p.) followed by isotonic saline (1 ml/kg, s.c.) 15 min later. Group 3 received isotonic saline (1 ml/kg, i.p.) followed by either 5-HTP (25 mg/kg, s.c.) or serotonin (2 mg/kg, s.c.) 15 min later. Group 4 received methysergide (3 mg/kg, i.p.) followed by either 5-HTP (25 mg/kg, s.c.) or serotonin (2 mg/kg, s.c.) 15 min later. Water intake was measured as described for Experiment 2.

Drugs

The 5-hydroxytryptamine creatinine sulfate complex (serotonin) was purchased from Sigma Chemical Co. The *l*-5-hydroxytryptophan monohydrochloride was purchased from Calbiochem-Behring Corp. Dr. C. Kadzielawa of the Department of Pharmacology and Therapeutics, University of Florida, College of Medicine generously donated the benserazide (RD 4-4602). The carbidopa was a gift from Dr. C. A. Stone of Merck, Sharpe, and Dohme Research Laboratories, West Point, PA. The methysergide maleate was a gift from Dr. C. E. Eden of Sandoz Pharmaceuticals, East Hanover, NJ.

Statistical Analysis

All data are shown as the mean and standard error. The data obtained from Experiment 1 were analyzed statistically by means of an analysis of variance [5]. Data obtained from all remaining experiments were analyzed statistically by means of an analysis of variance for a factorially designed experiment [5]. Differences between group means were analyzed statistically by means of a *t*-test using the pooled variance from the analysis of variance [5].

RESULTS

Experiment 1

Water intake of rats administered serotonin (0.0, 0.25, 0.5, 1, 2, or 4 mg/kg, s.c.) was increased in a dose-dependent manner after 0.5, 1, 2, and 3 hr. The dose-response curve for serotonin-induced water intake after 2 hr is shown in Fig. 1A. A similar dose-response pattern was found at the other times (0.5, 1, and 3 hr) water intake was measured. The time course of the serotonin (2 mg/kg, s.c.)-induced dipsogenic response is shown in Fig. 1B. This pattern is typical of that found for the other concentrations of serotonin (0.25, 0.5, 1, and 4 mg/kg, s.c.) tested. A highly significant increase in water intake was obtained when 2 mg serotonin/kg was administered subcutaneously (Fig. 1A). For this reason, this concentration of serotonin was used for all remaining experiments, except when testing for an interaction with 5-HTP, in which case 1 mg serotonin/kg was used. The dipsogenic response to serotonin appeared to reach a plateau by 2 hr after administration (Fig. 1B); therefore, for all remaining experiments, the 2nd hour data will be shown.

Experiment 2

Previous studies from this laboratory [10,18] have shown that administration of 5-HTP induces a large dipsogenic response in rats. In the present study, the central and peripheral decarboxylase inhibitor, benserazide (30 mg/kg, s.c.), abolished the 2 hr 5-HTP (25 mg/kg, s.c.)-induced water intake (Fig. 2A) but did not affect the serotonin (2 mg/kg, s.c.)-induced thirst, over the same time period (Fig. 2B). Similar responses were obtained 1 and 3 hr after administra-

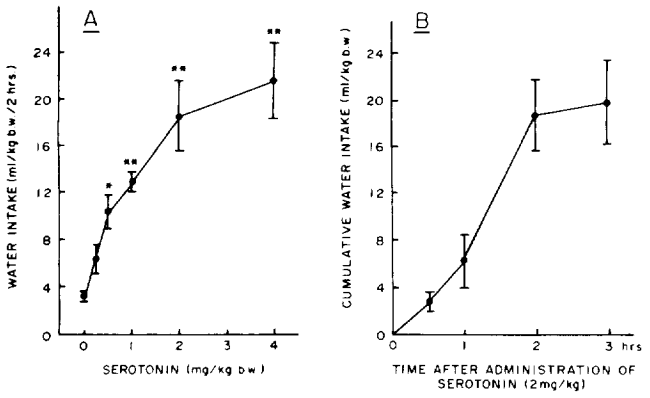

FIG. 1. Serotonin-induced changes in water intake: (A) Effect of graded doses of serotonin (0 to 4 mg/kg, s.c.) on cumulative water intake during the first 2 hr after administration. (B) Time-course (0 to 3 hr) of serotonin (2 mg/kg, s.c.)-induced water intake. Significant differences from control are denoted: $*p<0.05$, $**p<0.01$.

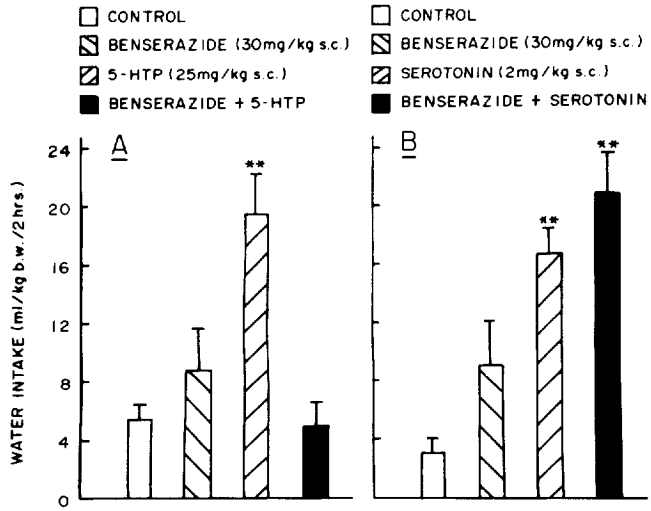

FIG. 2. Effect of pretreatment for 15 min with benserazide (30 mg/kg, s.c.) on the dipsogenic responses to 5-HTP (25 mg/kg, s.c.) (A) and serotonin (2 mg/kg, s.c.) (B). Cumulative water intake during the first 2 hr after administration of the dipsogens is shown. F-values: (A) 5-HTP, 5.04*; benserazide, 6.07*; interaction, 15.89**; (B) serotonin, 28.90**; benserazide, 4.76*; interaction, 0.15 (N.S.). Significant differences from control are denoted in the figure: $**p<0.01$.

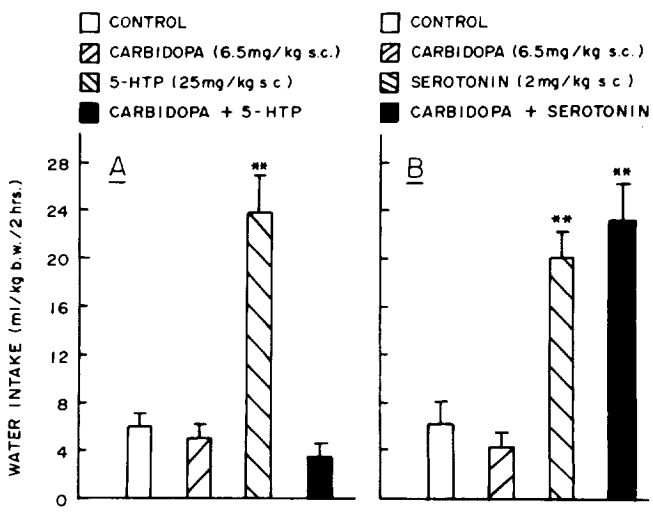

FIG. 3. Effect of pretreatment for 15 min with carbidopa (6.5 mg/kg, s.c.) on the dipsogenic responses to 5-HTP (25 mg/kg, s.c.) (A) and serotonin (2 mg/kg, s.c.) (B). Cumulative water intake during the first 2 hr after administration of the dipsogens is shown. F-values: (A) 5-HTP, 21.06**; carbidopa, 36.41**; interaction, 29.44**; (B) serotonin, 54.41**; carbidopa, 0.13 (N.S.); interaction, 1.10 (N.S.). Significant differences from control are denoted in the figure: $**p<0.01$.

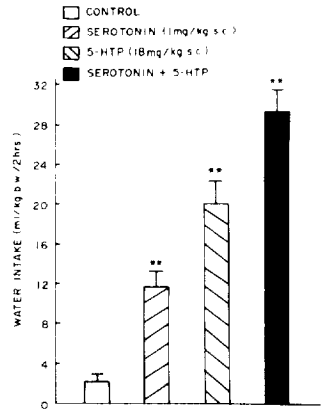

FIG. 4. Interaction between 5-HTP (18 mg/kg, s.c.) and serotonin (1 mg/kg, s.c.) on water intake. Cumulative water intake during the first 2 hr after administration of the dipsogens is shown. F-values: serotonin, 27.34**; 5-HTP, 98.95**; interaction, 0.01 (N.S.). Significant differences from control are denoted in the figure. $**p<0.01$.

tion of the dipsogenic agents. The peripheral decarboxylase inhibitor, carbidopa (6.5 mg/kg, s.c.), also abolished the 2 hr 5-HTP-induced water intake (Fig. 3A) without affecting the response to serotonin (Fig. 3B). Again, the same pattern of responses was seen 1 and 3 hr after administration of the dipsogenic agents.

Experiment 3

This experiment was designed to test for an interaction between 5-HTP and serotonin on water intake in the rat; therefore, one group of rats received both dipsogenic agents. The concentrations of 5-HTP and serotonin used in the last experiment induced almost maximal water intake; therefore, submaximal concentrations of the dipsogens (Fig. 1A) [10] were used for this experiment in order to detect any increase in water intake upon administration of both dipsogenic agents simultaneously. Rats receiving serotonin (1 mg/kg, s.c.) and those receiving 5-HTP (18 mg/kg, s.c.) showed significant increases in water intake after 2 hr (Fig. 4). Rats receiving both dipsogenic agents showed no interaction on water intake between the two compounds but rather a dipsogenic response approximately equal to the sum of the two

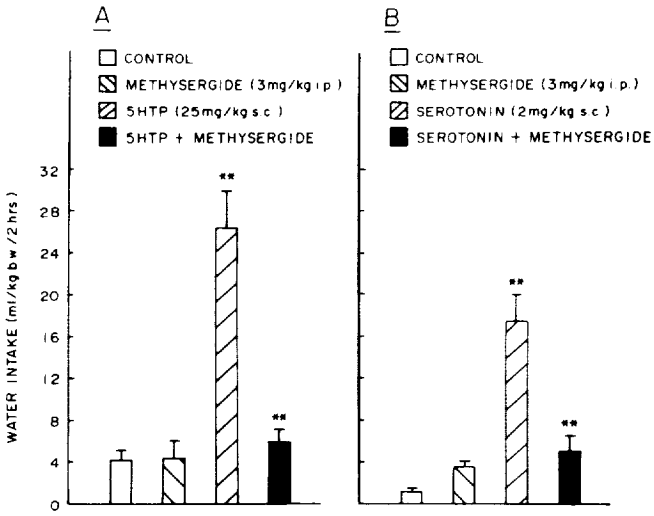

FIG. 5. Effect of pretreatment for 15 min with methysergide (3 mg/kg, i.p.) on the dipsogenic responses to 5-HTP (25 mg/kg, s.c.) (A) and serotonin (2 mg/kg, s.c.) (B). Cumulative water intake during the first 2 hr after administration of the dipsogens is shown. F-values: (A) 5-HTP, 30.02**; methysergide, 22.48**; interaction, 23.05**; (B) serotonin, 39.07**; methysergide, 12.02**; interaction, 26.11**. Significant differences from control are denoted in the figure: $**p<0.01$.

individual responses (Fig. 4). Similar responses were obtained 1 and 3 hr after administration of the dipsogenic agents.

Experiment 4

Fifteen min pretreatment with the peripheral serotonergic receptor antagonist, methysergide (3 mg/kg, i.p.), abolished the 2 hr dipsogenic response to both 5-HTP (25 mg/kg, s.c.) (Fig. 5A) and serotonin (2 mg/kg, s.c.) (Fig. 5B). The same pattern of responses was seen 1 and 3 hr after administration of the dipsogenic agents.

DISCUSSION

Serotonin stimulated drinking in rats (Fig. 1A) within the first 2 hr after administration (Fig. 1B), as previously reported by Meyer *et al.* [14]. This dipsogenic response was also similar to that reported previously for 5-HTP [10], the immediate precursor of serotonin [13]. Inhibition of the decarboxylation of 5-HTP to serotonin, centrally and peripherally with benserazide [3] (Fig. 2), as well as only peripherally with carbidopa [20] (Fig. 3), abolished the 5-HTP-induced drinking response without affecting the dipsogenic response to serotonin. This finding suggests that 5-HTP must be decarboxylated to serotonin, at a peripheral site in the body, in order to induce drinking.

The drinking response to 5-HTP in rats [18] is associated with an increased release of renin from the kidney [2]. Furthermore, serotonin has been shown to increase plasma renin concentration in the rat [2,14]. Inhibition of the decarboxylation of 5-HTP to serotonin, centrally and peripherally with benserazide, as well as only peripherally with carbidopa, abolished the 5-HTP-induced increase in plasma renin activity in the rat [2]. Therefore, peripheral decarboxylation of 5-HTP to serotonin may lead to an increase in plasma renin activity and, ultimately, to an increase in water intake. However, in contrast to these data obtained from the rat, Ganong *et al.* [11] and Zimmermann and Ganong [21] have reported that central, rather than peripheral, decarboxylation of 5-HTP to serotonin leads to an increase in plasma renin activity in the dog. If the dipsogenic response to serotonin is initiated by a release of renin, a species difference appears to exist in the site of production of serotonin which initiates renin release.

There was no interaction between 5-HTP and serotonin on the dipsogenic response when both were administered simultaneously (Fig. 4). In fact, the volume of water ingested after administration of the two compounds simultaneously was approximately equal to the sum of the dipsogenic responses to the two compounds when administered separately. These results suggest that 5-HTP induces a drinking response through formation of endogenous serotonin, which acts by the same mechanism as exogenous serotonin administered peripherally. An interaction between these two compounds would have negated this possibility.

The peripheral serotonergic receptor antagonist, methysergide [1,9], inhibited the drinking response to both 5-HTP (Fig. 5A) and serotonin (Fig. 5B). These data indicate that the dipsogenic activity of serotonin, whether endogenous or exogenous, is dependent on activation of peripheral serotonergic receptors. These results are in agreement with those of Meyer *et al.* [14] in which methysergide was shown to inhibit serotonin-induced drinking in rats. In addition they found that serotonin did not produce an increase in plasma renin concentration in the presence of methysergide. Therefore, serotonin probably induces thirst by activating peripheral serotonergic receptors which leads to an increase in renin release from the kidney.

This finding that serotonin induces thirst through a peripheral site of action in rats differs from the findings in other animals [15,21] in which serotonin acts centrally as a dipsogen. In the dog [21], blockade of central serotonergic receptors inhibited the 5-HTP-induced drinking response. In the monkey [15], injection of serotonin into the hypothalamus produced a dipsogenic response. However, no change in water intake was observed in rats injected intrahypothalamically with serotonin [17]. Recently van de Kar *et al.* [19] found that administration of serotonin-depleting agents to rats induced similar changes in both hypothalamic serotonin concentration and plasma renin activity. However, they did not study the effect of these agents on peripheral sources of serotonin.

Although these data indicate that activation of peripheral serotonergic receptors leads ultimately to an increased water intake in the rat, the pathway(s) by which activation of these receptors leads to the dipsogenic response is not known. As previously discussed, serotonin probably induces a drinking response in the rat via the renin-angiotensin system. Previous studies with rats [18] have shown that the dipsogenic response to 5-HTP, which is associated with an increase in plasma renin activity [2] is inhibited by blockade of β-adrenoceptors with propranolol and by inhibition of angiotensin converting enzyme with captopril. Meyer *et al.* [14] found that propranolol abolished the serotonin-induced increases in water intake and in plasma renin concentration in rats. Therefore, serotonin may increase drinking in rats by activating β-adrenergic receptors on juxtaglomerular cells which, in turn, increase release of renin from the kidney [11,16]. A decrease in blood pressure can also increase renin release through release of catecholamines from renal nerves

which activate the renal β-adrenoceptors [16]. In the rat, serotonin has been shown to have a hypotensive action [4]. This hypotensive action of serotonin could lead ultimately to an increase in water intake. However, this laboratory [2] reported recently that administration of 5-HTP (25 mg/kg, s.c.) to rats was not accompanied by a change in blood pressure while administration of serotonin (2 mg/kg), s.c.) induced a hypotensive response. If 5-HTP must be converted to serotonin in order to increase both water intake and plasma renin activity, then these responses are probably not due to changes in blood pressure unless 5-HTP initiates an effect which may mask the decrease in blood pressure, e.g. by release of catecholamines. Further studies will be necessary to determine how serotonin activates the renin-angiotensin system and initiates dipsogenesis.

ACKNOWLEDGEMENTS

The authors thank Mrs. Charlotte Edelstein for the graphic illustrations and Mr. Juan Posada for technical assistance.

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